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Stabilization of thylakoid membranes in isoprene-emitting plants reduces formation of reactive oxygen species

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Isoprene is emitted by a significant fraction of the world's vegetation. Isoprene makes leaves more thermotolerant, yet we do not fully understand how. We have recently shown that isoprene stabilizes thylakoid membranes under heat stress. Here we show that heat-stressed, isoprene-emitting transgenic Arabidopsis plants also produce a lower pool of reactive oxygen and reactive nitrogen species, and that this was especially due to a lower accumulation of H_2O_2 in isoprene emitting plants. It remains difficult to disentangle whether in heat stressed plants isoprene also directly reacts with and quenches reactive oxygen species (ROS), or reduces ROS formation by stabilizing thylakoids. We present considerations that make the latter a more likely mechanism, under our experimental circumstances.

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Biogenic isoprene emission results sometimes in a very large loss of carbon and energy from plants, especially under stress conditions when carbon uptake is largely inhibited.[1] Thus, it is likely that isoprene biosynthesis provides some benefit to plants to balance the negative selection pressure that would come from such a large loss of energy and carbon. In the search for isoprene function, two theories have received increasing attention. On one side, isoprene was thought to stabilize membranes. This seems to be predominantly related to the lipophylic properties of isoprene and its ability to intercalate into membranes and strengthen them under conditions that make them fragile, especially heat stress.[2] Earlier research indeed demonstrated that isoprene-emitting plants are more tolerant to heat stress.[3-7] On the other side, isoprene was thought to quench reactive oxygen species (ROS) inside leaves, therefore indirectly providing a general antioxidant action that could be seen under multiple stresses.[8] This would explain why isoprene also protects leaves from strongly oxidative species such as ozone[9-11] or singlet oxygen.[12,13]

Velikova et al.,[14] using several different biophysical techniques, directly assessed the impact of isoprene on thylakoid intactness and functionality. Circular dichroism measurements indicated that isoprene emission allowed macrodomains of thylakoid membranes with embedded photosystem II complexes to remain stable when exposed to temperatures 5–8°C higher in isoprene-emitting Arabidopsis plants than in non-emitting plants. The position of the main thermoluminescence peak (Q_B peak) in isoprene-emitting plants was shifted up by about 10°C suggesting modifications in the lipid environment due to the presence of isoprene. Here ancillary data from this recent work[14] are presented. We show that isoprene-emitting transgenic Arabidopsis plants accumulated less ROS and similar amount reactive nitrogen species (RNS) in comparison to non-emitting wild-type plants, in response to heat stress.

Transgenic plants are great tools to study the impact of single traits on whole plant physiology. Arabidopsis plants were transformed by one of us to introduce the gene encoding for isoprene synthase, the enzyme that make isoprene from its substrate, dimethylallyl diphosphate, DMADP.[15] The amounts of NO and

H_2O_2, two key molecules in plant signaling inducing hypersensitive responses to stress,[16,17] were measured in wild-type and *IspS* plants before stress at growth temperature (22°C) and after exposure to heat (38°C; **Fig. 1**) for 48 h. A significant amount of research has been recently devoted to unravel the possible interactions of NO with ROS, and how these interactions can modulate the signaling of stress responses.[19,20] Maintenance of physiological ROS levels is essential to ensure an accurate execution of their signaling functions, and to prevent their toxicity. Therefore, plants have evolved an antioxidant system composed of enzymatic and non-enzymatic antioxidants to harmlessly scavenge excessive ROS production.[21] However, when plants are exposed to unfavorable environmental conditions a rapid increase in ROS and RNS production (the so called "oxidative burst") is often induced.[22] Indeed, a significant increase of H_2O_2, and NO was found in both wild-type and isoprene-emitting heat-stressed Arabidopsis plants (**Fig. 1**). However, the accumulation of H_2O_2 was substantially higher in wild-types than in *IspS* lines, whereas NO increased similarly in the two plants compared with pre-stress conditions.

By changing the balance between NO and H_2O_2 under heat stress conditions, isoprene emission may also modify the signaling pattern of these molecules.[16] When isoprene is not produced, the simultaneous accumulation of H_2O_2 and NO may trigger cell death signaling, whereas in isoprene-emitting plants the accumulation of H_2O_2 and NO may not reach toxic levels or their ratio may not be adequate. Consequently, the signaling action of the two molecules might be dimmed and hypersensitive responses might be prevented.

Why is only H_2O_2 accumulation reduced in *Isps* plants? In past experiments with plants naturally emitting isoprene the amount of both H_2O_2[9] and NO[23] was reduced and it was suggested that isoprene could directly react with ROS and RNS.[8] However, we note that: i) NO accumulation was found to be reduced in isoprene-emitting plants compared with isoprene-emitting plants exposed to ozone,[23] but may not be affected in response to a stress that does not involve direct oxidation of the medium, as in our heat stress experiment; ii) NO is not a very reactive molecule and the rate constant of its reaction with isoprene would require a large concentration of isoprene in the leaves for the reaction to occurs.[1] Such a large isoprene concentration may be found in natural, strong emitters but not in transgenic Arabidopsis that emit ten times less than strong emitters[14]; iii) the attenuated increase of H_2O_2 in *IspS* lines may not directly involve H_2O_2 reactivity with, and consequent quenching by isoprene. Lower accumulation of H_2O_2 may be an indirect consequence of preserved functionality of photosynthetic regulatory mechanisms in *IspS* lines, in turn due to isoprene-induced membrane stabilization.[14] If the photochemical regulatory apparatus is preserved, then the photosynthetic electron transport rate runs more efficiently as we already demonstrated,[14] and photochemical energy is not dissipated through direct oxygen photoreduction.[24] Thus the lower H_2O_2 generation might be due to less oxygen photoreduction and consequently less generation of dangerous levels of ROS.[25]

Isoprene general protection against environmental stresses is well known.[8] Theoretically, isoprene may both strengthen membranes[26] and quench reactive compounds, predominantly reactive oxygen species.[8] It might be argued that heat stress specifically affects membrane stability, and thus should make isoprene protection particularly evident if isoprene only strengthens membranes. However, the relationship between ROS and membrane stability is shown in a cartoon in **Figure 2**. If ROS damage membranes and damaged membranes lead to ROS production then a feedforward loop can occur. Stresses that make ROS (e.g., ozone) and stresses that damage membranes (e.g., heat) will activate the feedforward cycle and lead to H_2O_2 accumulation and eventually cell death. Isoprene could stop this feedforward cycle in either of two ways: (1) quenching the ROS, and (2) stabilizing membranes. If isoprene quenches ROS the products of the isoprene/ROS interaction need to be considered. One prominent product is methylvinyl ketone, which could be cytotoxic.[27] The results of Velikova et al.[14] allow speculation that membrane stabilization is a major mechanism by which isoprene helps plants tolerate abiotic stress of many different forms.

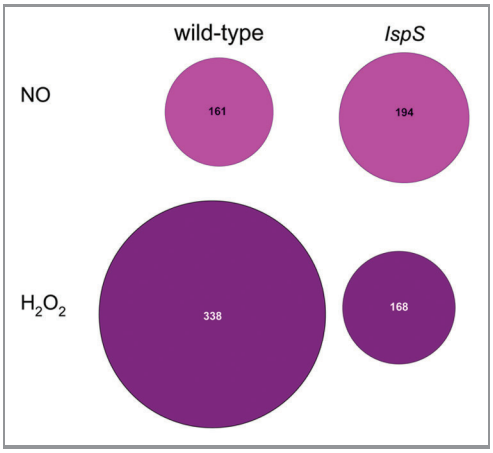

Figure 1. Increase of NO (pink) and H_2O_2 (purple) contents in wild-type (non-emitting) and transgenic *IspS* (isoprene-emitting) Arabidopsis plants after exposure to heat (38°C for 48 h). Values are percent increase with respect to pools of NO and H_2O_2 detected in wild-type and *IspS* plants grown at 22°C. Details on measurements of NO emission and calculation of NO concentration inside the leaves, and on determination of H_2O_2 content are described in Velikova et al.[18]

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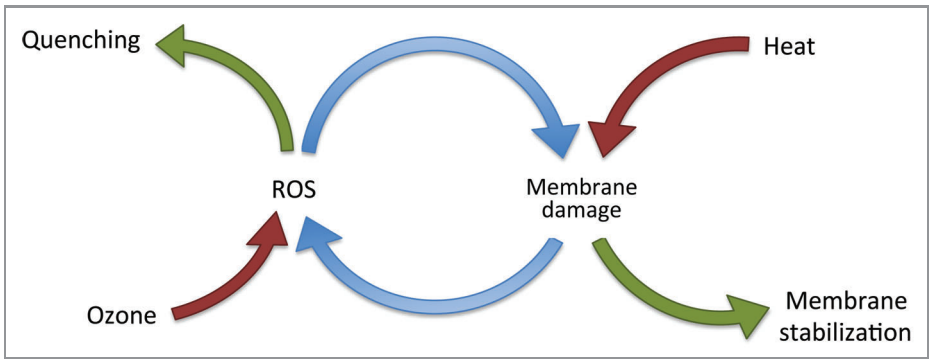

Figure 2. Simplified diagram showing two possible effects of isoprene (in green). If there is a central cycle in which ROS leads to membrane damage and membrane damage provides a signal for production of ROS (in blue), this feedforward loop could be initiated by many different stresses (e.g., ozone or heat stress) and could also be stopped by several different mechanisms (e.g., membrane stabilization or quenching of ROS). Isoprene is thought to lead to both, and indeed membrane are strengthened[14] and ROS are quenched (this work, **Figure 1**) in genetically modified *IspS* Arabidopsis producing isoprene and exposed to heat stress. However, because heat stress primarily impinges on membranes, the demonstration by Velikova et al.[14] that significant membrane stabilization is conferred by isoprene allows speculation that quenching of ROS is a side effect, and does not account for the protective effects observed for isoprene in heat stressed plants.

References

1. Loreto F, Schnitzler J-P. Abiotic stresses and induced BVOCs. Trends Plant Sci 2010; 15:154-66; PMID: 20133178; http://dx.doi.org/10.1016/j.tplants.2009.12.006
2. Sharkey TD, Yeh S. Isoprene emission from plants. Annu Rev Plant Physiol Plant Mol Biol 2001; 52:407-36; PMID:11337404; http://dx.doi.org/10.1146/annurev.arplant.52.1.407
3. Sharkey TD, Singsaas EL. Why plants emit isoprene. Nature 1995; 374:769; http://dx.doi.org/10.1038/374769a0
4. Singsaas EL, Lerdau M, Winter K, Sharkey TD. Isoprene increases thermotolerance of isoprene-emitting species. Plant Physiol 1997; 115:1413-20; PMID: 12223874
5. Hanson DT, Swanson S, Graham LE, Sharkey TD. Evolutionary significance of isoprene emission from mosses. Am J Bot 1999; 86:634-9; PMID:10330065; http://dx.doi.org/10.2307/2656571
6. Peñuelas J, Llusià J, Asensio D, Munné-Bosch S. Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. Plant Cell Environ 2005; 28:278-86; http://dx.doi.org/10.1111/j.1365-3040.2004.01250.x
7. Velikova V, Loreto F. On the relationship between isoprene emission and thermotolerance in Phragmites australis leaves exposed to high temperatures and during the recovery from a heat stress. Plant Cell Environ 2005; 28:318-27; http://dx.doi.org/10.1111/j.1365-3040.2004.01314.x
8. Vickers CE, Gershenzon J, Lerdau MT, Loreto F. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nat Chem Biol 2009; 5:283-91; PMID:19377454; http://dx.doi.org/10.1038/nchembio.158
9. Loreto F, Velikova V. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol 2001; 127:1781-7; PMID:11743121; http://dx.doi.org/10.1104/pp.010497
10. Loreto F, Fares S. Is ozone flux inside leaves only a damage indicator? Clues from volatile isoprenoid studies. Plant Physiol 2007; 143:1096-100; PMID: 17344434; http://dx.doi.org/10.1104/pp.106.091892
11. Vickers CE, Possell M, Cojocariu CI, Velikova VB, Laothawornkitkul J, Ryan A, et al. Isoprene synthesis protects transgenic tobacco plants from oxidative stress. Plant Cell Environ 2009; 32:520-31; PMID: 19183288; http://dx.doi.org/10.1111/j.1365-3040.2009.01946.x
12. Affek HP, Yakir D. Protection by isoprene against singlet oxygen in leaves. Plant Physiol 2002; 129:269-77; PMID:12011357; http://dx.doi.org/10.1104/pp.010909
13. Velikova V, Edreva A, Loreto F. Endogenous isoprene protects *Phragmites australis* leaves against singlet oxygen. Physiol Plant 2004; 122:219-25; http://dx.doi.org/10.1111/j.0031-9317.2004.00392.x
14. Velikova V, Várkonyi Z, Szabó M, Maslenkova L, Nogues I, Kovács L, et al. Increased thermostability of thylakoid membranes in isoprene-emitting leaves probed with three biophysical techniques. Plant Physiol 2011; 157:905-16; PMID:21807886; http://dx.doi.org/10.1104/pp.111.182519
15. Sharkey TD, Yeh S, Wiberley AE, Falbel TG, Gong D, Fernandez DE. Evolution of the isoprene biosynthetic pathway in kudzu. Plant Physiol 2005; 137:700-12; PMID:15653811; http://dx.doi.org/10.1104/pp.104.054445
16. Delledonne M, Zeier J, Marocco A, Lamb C. Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. Proc Natl Acad Sci USA 2001; 98:13454-9; PMID:11606758; http://dx.doi.org/10.1073/pnas.231178298
17. Delledonne M.. NO news is good news for plants. Curr Opin Plant Biol 2005; 8:390-6; PMID:15922651; http://dx.doi.org/10.1016/j.pbi.2005.05.002
18. Velikova V, Fares S, Loreto F. Isoprene and nitric oxide reduce damages in leaves exposed to oxidative stress. Plant Cell Environ 2008; 31:1882-94; PMID: 18811730; http://dx.doi.org/10.1111/j.1365-3040.2008.01893.x
19. Lamattina L, García-Mata C, Graziano M, Pagnussat G. Nitric oxide: the versatility of an extensive signal molecule. Annu Rev Plant Biol 2003; 54:109-36; PMID:14502987; http://dx.doi.org/10.1146/annurev.arplant.54.031902.134752
20. Neill SJ, Desikan R, Hancock JT. Nitric oxide signaling in plants. New Phytol 2003; 159:11-35; http://dx.doi.org/10.1046/j.1469-8137.2003.00804.x
21. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Reactive oxygen gene network of plants. Trends Plant Sci 2004; 9:490-8; PMID:15465684; http://dx.doi.org/10.1016/j.tplants.2004.08.009
22. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 2004; 55:373-99; PMID:15377225; http://dx.doi.org/10.1146/annurev.arplant.55.031903.141701
23. Velikova V, Pinelli P, Pasqualini S, Reale L, Ferranti F, Loreto F. Isoprene decreases the concentration of nitric oxide in leaves exposed to elevated ozone. New Phytol 2005; 166:419-25; PMID:15819906; http://dx.doi.org/10.1111/j.1469-8137.2005.01409.x
24. Laisk A, Loreto F. Determining photosynthetic parameters from leaf CO2 exchange and chlorophyll fluorescence: Rubisco specificity factor, dark respiration in the light, excitation distribution between photosystems, alternative electron transport and mesophyll diffusion resistance. Plant Physiol 1996; 110:903-12; PMID:12226229
25. Asada K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 2006; 141:391-6; PMID:16760493; http://dx.doi.org/10.1104/pp.106.082040
26. Siwko ME, Marrink SJ, de Vries AH, Kozubek A, Schoot Uiterkamp AJM, Mark AE. Does isoprene protect plant membranes from thermal shock? A molecular dynamics study. Biochim Biophys Acta 20071768; 198-206; PMID:17125733
27. Vollenweider S, Weber H, Stolz S, Chételat A, Farmer EE. Fatty acid ketodienes and fatty acid ketotrienes: Michael addition acceptors that accumulate in wounded and diseased Arabidopsis leaves. Plant J 2000; 24:467-76; PMID:11115128; http://dx.doi.org/10.1046/j.1365-313x.2000.00897.x